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Transvascular Catheter Infiltration of the Ductus Arteriosus With Formalin in the Newborn Lamb: A Novel Method of Establishing and Maintaining Ductal Patency

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Objectives. **This study sought to establish and maintain patency of the ductus arteriosus by a new method of transvascular formalin infiltration of the ductus arteriosus wall.**

Background. **Maintaining patency of the ductus arteriosus postnatally is necessary with many forms of congenital heart disease to ensure survival until definitive surgical repair. A variety of approaches have to date met with variable success.**

Methods. **Seven newborn lambs underwent catheterization at 1 to 5 days of age. A functionally closed ductus arteriosus was traversed with a specially designed porous balloon catheter, and the wall was infiltrated with 10% formalin. The ductus was then further dilated with either a 7- or 8-mm diameter balloon.**

Results. **The ductus arteriosus wall was successfully infiltrated and dilated in all animals. Two lambs were euthanized within 24 h with congestive heart failure from a large ductus, and one lamb was electively euthanized 5 days after transvascular infiltration. Four lambs underwent serial follow-up catheterizations, one of which required repeat balloon dilation 47 days after infiltration. At latest follow-up (mean age [±1 SD] 83 ± 34 days, range 33 to 108), the pulmonary/systemic flow ratio (2.7 ± 1.2) was unchanged from immediately after infiltration (1.9 ± 0.5, $p > 0.1$). The narrowest diameter of the ductus arteriosus (4.3 ± 0.4 mm vs. 6.9 ± 2.6 mm, $p > 0.1$) and its ratio to that of the adjacent descending aorta (0.5 ± 0.1 vs. 0.4 ± 0.1, $p > 0.1$) were also unchanged at latest follow-up. No systemic toxicity was observed. At postmortem study, the ductus was patent, and histologic analysis showed variable intimal and medial destruction, endothelial regeneration, loss of elastic tissue and calcification. Adjacent pulmonary artery and aorta were normal. Based on the small sample size and the observed maximal effect size of 70%, the power of the study is at most 40% to detect significant differences.**

Conclusions. **To our knowledge, this is the first time that transvascular formalin infiltration of the ductus arteriosus wall has been applied successfully to maintain ductal patency in the newborn lamb. This method may provide a means of palliation for neonates with certain forms of congenital heart disease.**

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A number of investigators have attempted procedures to establish and maintain patency of the ductus arteriosus. Balloon dilation (1) has been uniformally unsuccessful; endovascular stenting (2) can lead to embolization of the stent or spontaneous thrombosis; and radiofrequency thermal balloon angioplasty (3) of the ductus leads to progressive stenosis during follow-up. Before the advent of the recent era of pharmacologic management with prostaglandin E_1, Rudolph et al. (4) demonstrated that surgical (open-chest) infiltration of the patent ductus arteriosus with formalin led to sustained ductal patency and adequate pulmonary blood flow in infants with ductus-dependent cyanotic congenital heart disease (4). We have evaluated the feasibility of a new method for establishing and maintaining patency of the ductus arteriosus in the closed-chest newborn lamb by infiltrating and dilating the ductal wall with formalin using a custom designed radially perforated balloon catheter.

Methods

Catheterization technique. This study was approved by the animal use committee of the Pennsylvania State University Medical Center and conforms to the position of the American Heart Association on research use. Seven term newborn lambs underwent cardiac catheterization at 1 to 5 days of age (weight 5.8 ± 1.1 kg, range 3.9 to 7.2). The lambs were sedated with intramuscular ketamine (5 to 10 mg/kg). The external jugular vein was cannulated percutaneously under local anesthesia with xylocaine, and a continuous infusion of ketamine (0.1 to 0.2 mg/kg body weight per h) and fentanyl (1 μg/kg per h) was utilized for sedation. Atropine (0.01 mg/kg) was administered to minimize secretions. Size 6F sheaths were placed percuta-

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Figure 1. Aortic angiogram before infiltration demonstrates a closed ductus arteriosus. **Solid white arrow** indicates level of ductus arteriosus. Ao = aorta.

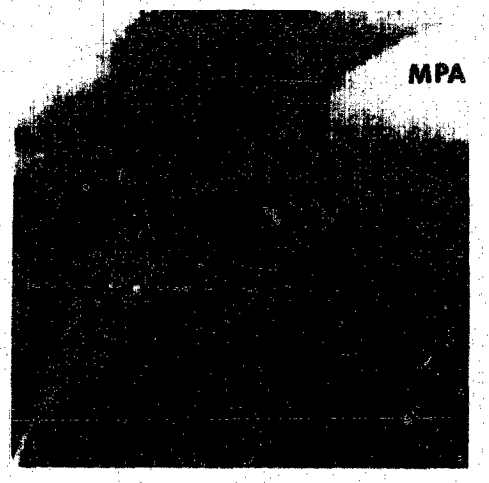

Figure 2. Aortic angiogram (lamb 5) 1 h after infiltration demonstrates a widely patent ductus arteriosus (D) measuring 3.9 mm at its narrowest point (ductus/descending aorta ratio 0.45) and opacification of the main pulmonary artery (MPA). **Dashed black line** indicates level (immediately below ductus) where the descending aorta measurement was taken.

neously into the right femoral artery and vein under local anesthesia. Prophylactic antibiotics (ampicillin 100 mg/kg and gentamycin 1.5 mg/kg) were administered following cannulation, at the conclusion of the procedure and twice daily for 48 h. The spontaneously breathing supine lamb was catheterized under fluoroscopy. Catheter patency was maintained by a constant pump infusion (3 ml/h) of heparinized 5% dextrose solution. Temperature, respiration, blood hematocrit and arterial blood gases were periodically monitored.

Hemodynamic measurements. Heart rate and hemodynamic pressures were recorded using a multichannel physiologic recorder and fluid-filled cardiac catheters. Baseline right heart, distal left pulmonary artery and wedge pressure recordings, blood gases and oximetry were measured to detect a left-to-right cardiac shunt (pulmonary to systemic flow ratio >1.0). The superior vena cava and right ventricular hemoglobin oxygen saturations and Po_2 were identical in all studies, indicating no atrial level left-right shunting, and thus the superior vena cava saturation was utilized to calculate the pulmonary to systemic flow ratio according to the standard formula: Qp/Qs = (Aortic oxyhemoglobin saturation − Superior vena cava saturation) ÷ (Aortic saturation − Distal left pulmonary artery saturation). Left ventricular and aortic pressure were measured by retrograde catheter passage from the femoral artery. Imaging of the aortic arch and ductus arteriosus was accomplished by cineangiocardiography using pressure-injected contrast and cine recording at 60 frames/s.

Ductus infiltration technique. An aortic angiogram in a 90° lateral view was obtained to optimally visualize and assess the location of the ductus arteriosus (Fig. 1). A 5F right coronary catheter was then advanced retrogradely from the femoral artery to the presumed level of the ductus arteriosus. The functionally closed ductus arteriosus was easily recannulated with a soft-tipped 0.035-in. (0.89-mm) diameter guide wire. A 4.3F triple-lumen polyvinyl balloon catheter perforated with six longitudinal rows containing 50 perforations of 25-μm diameter (4-mm diameter × 2-cm balloon length) was advanced across the recanalized ductus arteriosus. The perforated region of the balloon (middle 1 cm) was positioned within the constricted ductus lumen, which was then infiltrated along its entire length. Approximately 3 ml of a 10% buffered formaldehyde (formalin) solution was injected using a 5-ml syringe under manual pressure that inflates the balloon and extravasates the formalin into the ductal wall. Three separate injections, each no more than 5 s in duration, were used to infiltrate the entire length of the ductus. Because the ductal diameter was initially small after transvascular formalin infiltration, we arbitrarily utilized a subsequent balloon dilation catheter of 7 mm (three lambs) or 8 mm (four lambs) diameter to further dilate the ductus.

One hour after the formalin infiltration, repeat hemodynamic pressures, blood gases and hemoglobin oxygen saturations were measured to estimate the pulmonary to systemic flow ratio with the ductus open. Two lambs were killed within 24 h after transvascular formalin infiltration and did not undergo serial follow-up catheterizations. Our initial experiment (lamb 1) underwent repeat catheterization at 5 days of age only, and four lambs were recatheterized on at least two occasions (early follow-up 18 ± 4 days of age, range 12 to 22; latest follow-up 83 ± 34 days of age, range 33 to 108). We obtained additional aortic angiograms 1 h following formalin infiltration (Fig. 2) and at each follow-up catheterization in order to confirm ductal patency and to measure the narrowest diameter of the ductus arteriosus and the adjacent descending aorta (Fig. 3). Liver enzymes (alanine aminotransferase, aspar-

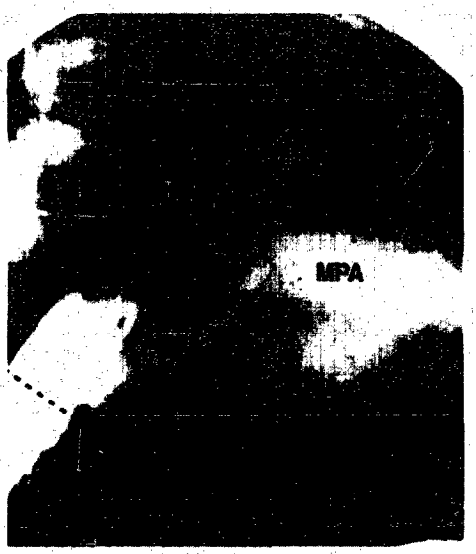

Figure 3. Aortic angiogram at 96 days of age (same animal as in Fig. 2) demonstrates a persistently patent ductus arteriosus now measuring 6.4 mm at its narrowest point and an unchanged ductus/descending aorta ratio of 0.43. T = tracheal air shadow; other abbreviations as in Figure 2.

tate aminotransferase and alkaline phosphatase), hemoglobin, urea nitrogen and creatinine were measured by the pathology laboratory within the Department of Veterinary Medicine at the Pennsylvania State University hospital before infiltration, 1 h after infiltration and at each follow-up catheterization.

Postmortem evaluation. Two lambs with a large left-right shunt and congestive heart failure were humanely sacrificed 24 h following the procedure. The remaining five lambs were sacrificed, respectively, at 5, 33, 96, 96 and 108 days of age in accordance with the panel of euthanasia of the American Veterinary Medical Association. The ductus arteriosus, adjacent aorta and main pulmonary artery were excised en bloc, fixed in 10% buffered formalin and sectioned for histologic evaluation. All microscopic specimens were stained with hematoxylin and eosin and a Verhoff-Van Gieson elastic stain.

Statistics. Data are expressed as the mean ± 1 standard deviation. Four lambs had complete serial data (before, 1 h after and early and late follow-up) compared by analysis of variance for repeated measures followed by the Scheffé test for multiple intragroup comparisons. Based on the small sample size and the observed maximal effect size of 70%, the power of the study is at most 40% to detect significant differences. A p value <0.05 was considered significant.

Results

Angiography and hemodynamic variables. The preinfiltration aortic angiogram demonstrated a functionally closed ductus arteriosus in 7 lambs. In all, the ductus was successfully traversed, infiltrated and balloon dilated. Two lambs deteriorated rapidly with signs of a large ductus and heart failure and were sacrificed 24 h after the formalin infiltration. These were the smallest lambs (3.9 and 4.6 kg) in which we utilized a larger 8-mm diameter balloon to augment dilation of the ductus following formalin infiltration. The ductus arteriosus in both lambs was widely patent 1 h after infiltration and at postmortem. Our first experiment (lamb 1) underwent successful transvascular formalin infiltration and was intentionally sacrificed following early follow-up catheterization at 5 days of age. The remaining four lambs underwent repeat cardiac catheterizations from 12 to 108 days of age (Table 1).

Before transvascular formalin infiltration, no left-to-right shunting was evident by blood oximetry or Po_2 measurements (pulmonary to systemic flow ratio of 1.0 in all lambs). One hour after formalin infiltration, the pulmonary to systemic flow ratios were significantly increased and remained unchanged at last follow-up (1.9 ± 0.5 vs. 2.7 ± 1.2, $p > 0.1$). The narrowest diameter of the ductus arteriosus (4.3 ± 0.4 vs. 6.9 ± 2.6 mm, $p > 0.1$) and its ratio to that of the adjacent descending aorta (0.5 ± 0.1 vs. 0.4 ± 0.1, $p > 0.1$) did not change significantly at latest follow-up. The cineangiographic appearance of the ductus arteriosus was uniform, without discrete stenosis or filling defects. It is of interest that the ductus arteriosus in lamb 4 at 18 days of age was patent, with a pulmonary to systemic flow ratio of 1.6 (narrowest ductal diameter 3.8 mm, ratio 0.34), which was slightly greater than the ratio of 1.4 measured 1 h after formalin infiltration. In this lamb, the largest of the study (7.2 kg at birth), a smaller 7-mm diameter balloon catheter was utilized for ductus dilation following formalin infiltration. Forty-seven days after formalin infiltration (not listed in Table 1 or included in statistical analysis), the ductus in this lamb was uniformly constricted with no appreciable left-to-right shunt, but it was successfully redilated with an 8-mm diameter balloon. Approximately 2 months later (108 days of age), the pulmonary to systemic flow ratio was 1.7 (narrowest ductal diameter 4.1 mm, ratio 0.26). Mean left pulmonary artery pressure was similar before and 1 h after formalin infiltration (20 ± 4 vs. 32 ± 9 mm Hg, $p > 0.05$) and unchanged at follow-up catheterization. Mean left pulmonary artery wedge pressure increased significantly from before infiltration to latest follow-up (5 ± 1 vs. 12 ± 5 mm Hg, $p < 0.05$) consistent with increased pulmonary blood flow and volume loading of the left ventricle. Mean aortic pressure increased as expected with growth of the animals, whereas mean right atrial pressure was unchanged during follow-up. Left ventricular ejection was qualitatively normal. Before elective termination ($n = 5$), four lambs had loud continuous murmurs, and one had a systolic murmur.

Metabolic data. Except for the two lambs sacrificed at 24 h after infiltration, the remaining five lambs demonstrated good weight gain and no clinical evidence of congestive heart failure. Liver enzymes, arterial blood gases, hemoglobin and renal function were normal and unchanged 1 h after transvascular formalin infiltration and at each follow-up catheterization.

Table 1. Summary of Hemodynamic and Angiographic Findings

Age	Wt (kg)	Qp/Qs	Ductus (mm)	DA/AO	PAm (mm Hg)	AOm (mm Hg)	RAm (mm Hg)	LPAw (mm Hg)
Experiment 1								
1 day	4.2	1	NA	NA	24	92	4	5
1 h post-FI	—	4.1	4.3	0.47	22	90	4	8
5 days	4.2	2.6	7.2	0.95	42	80	5	21
Experiment 2								
5 days	6.3	1.0	NA	NA	16	75	2	3
1 h post-FI	—	2.7	4.1	0.41	26	75	2	4
12 days	8.5	1.8	5.8	0.58	18	82	8	10
33 days	15.3	2.0	6.9	0.43	18	115	9	14
Experiment 3								
5 days	6.1	1.0	NA	NA	16	84	4	6
1 h post-FI	—	2.0	4.4	0.52	26	84	2	12
19 days	10.1	2.0	6.2	0.57	24	84	7	10
108 days	25.2	2.6	10.3	0.53	24	112	6	18
Experiment 4								
3 days	7.2	1.0	NA	NA	22	100	3	5
1 h post-FI	—	1.4	4.9	0.48	45	80	4	6
18 days	10.9	1.6	3.8	0.34	12	96	5	11
96 days	22.5	1.7	4.1	0.26	16	112	3	9
Experiment 5								
4 days	5.3	1.0	NA	NA	24	88	6	4
1 h post-FI	—	1.7	3.9	0.45	32	68	3	8
22 days	8.3	3.1	5.8	0.46	22	74	5	16
96 days	16.7	4.3	6.4	0.43	13	84	2	8

AOm = mean aortic pressure; DA/AO = ratio of narrowest diameter of ductus arteriosus (DA) and adjacent descending aorta (AO); Ductus = narrowest diameter of the ductus arteriosus; FI = formalin infiltration; LPAw = mean left pulmonary artery wedge pressure; NA = not applicable; PAm = mean left pulmonary artery pressure; Qp/Qs = pulmonary/systemic blood flow ratio; RAm = mean right atrial pressure (mm Hg); Wt = weight.

Pathologic findings. Postmortem examination revealed a patent ductus arteriosus in all seven animals without intraluminal thrombosis or space-filling defects and consistent with the angiographic results. The two lambs sacrificed 24 h after transvascular formalin infiltration had significant cardiomegaly, pulmonary congestion and a large caliber ductus arteriosus. Pathologic examination of other organs was not performed, and therefore we can not entirely exclude formalin toxicity contributing to the symptoms recognized at the time of sacrifice. These two lambs and the animal electively sacrificed at 5 days of age demonstrated intimal destruction with fibrin deposition and focal necrosis of the media with scattered acute inflammatory cells within all layers of the vessel. The four remaining lambs showed varying degrees of intimal fibrosis with partial reconstitution of the endothelial layer (Fig. 4). Segmental areas of the media showed extensive destruction of elastic fibers and replacement by fibrous tissue with focal calcification and ossification. The constricted ductus that was redilated 47 days after transvascular formalin infiltration (lamb 4) demonstrated increased calcification, ossification and fibrosis in comparison to the other specimens. Adjacent aorta and main pulmonary artery were normal in all specimens.

Discussion

Maintaining patency of the ductus arteriosus postnatally is necessary in infants with congenital heart defects associated with inadequate systemic or pulmonary blood flow. Currently, this is accomplished using continuous intravenous prostaglandin E_1, which inhibits contraction of the smooth muscle cells within the wall of the ductus arteriosus. However, surgical palliation (constructing a systemic-pulmonary arterial shunt in infants with cyanotic congenital heart disease) is inevitably required. Consequently, a cardiac catheter technique that could lead to sustained and predictable ductal patency until definitive repair is optimal, would offer a central shunt potentially avoiding surgical complications and pulmonary artery distortion.

Experimental studies. Catheter methods previously attempted to reestablish and maintain ductus arteriosus patency include balloon dilation, radiofrequency thermal balloon angioplasty and stenting. Rosenthal et al. (1) reported that balloon dilation of the closed ductus arteriosus in 17 newborn lambs was universally ineffective. Spontaneous closure or significant constriction generally occurred within minutes after the procedure, indicating that the smooth muscle cells within the ductus arteriosus wall remained viable and capable of constricting spontaneously. In contrast, placement of an endovascular stent within the ductus arteriosus was effective with an acceptable pulmonary to systemic flow ratio immediately following the procedure. However, long-term assessment following ductal stenting is not available. Further potential disadvantages include the risk of device embolization and the requirement of anticoagulation to prevent thrombosis. Abrams

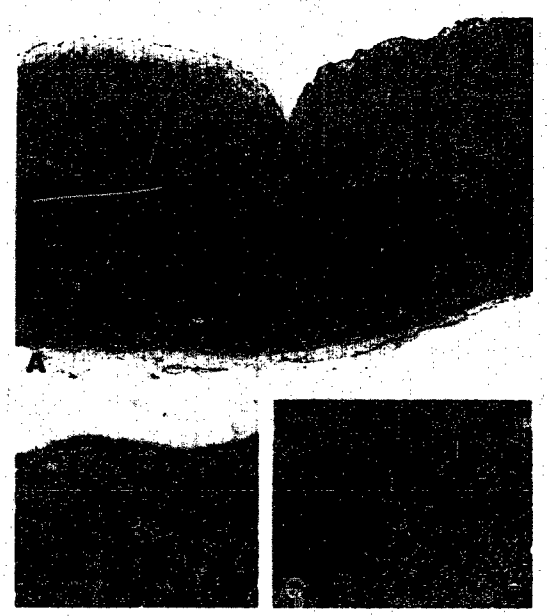

Figure 4. Histologic studies of ductus arteriosus at postmortem study (lamb 3; 108 days of age). **A,** The intima of the ductus (**arrow**) shows increased fibrosis. ×25. **B,** The endothelial layer is intact. Hematoxylin-eosin stain; ×150. **C,** Focal disruption of the internal elastic membrane is noted (**arrow**), and there is some loss of elastic tissue in the media. Verhoff-van Gieson elastic stain; ×40.

et al. (3) reported on the use of radiofrequency balloon angioplasty of the closed ductus arteriosus in 28 newborn lambs. Long-term ductal patency (46 ± 28 weeks) was maintained in 80% of the animals, and 20% ultimately closed. Ductal diameter in fact decreased and stenosis developed in all lambs at follow-up catheterizations, which is in contrast to what we observed in three of our long-term studies of lambs following transvascular formalin infiltration.

In 1974, Lakier et al. (5) reported that surgical infiltration of the ductus arteriosus of late gestational lambs with formaldehyde prevented spontaneous closure postnatally without any apparent adverse effects. Rudolph et al. (4) subsequently utilized this concept in four newborn infants with cyanotic congenital heart disease. The ductus arteriosus was exposed at thoracotomy and infiltrated with 10% buffered formaldehyde. All four infants improved clinically with a rise in systemic arterial saturation consistent with increased pulmonary blood flow that was maintained in three of the infants for 1, 5 and 9 months, respectively. No local or systemic toxicity was reported.

Formalin toxicity. Formaldehyde is a highly reactive substance that can interact with virtually every cellular constituent. Liver and other tissues rapidly metabolize formaldehyde to formic acid and subsequently to carbon dioxide following a reaction with glutathione or tetrahydrofolic acid. It is an irritant that produces local effects and may lead to systemic toxicity by producing organotropic effects on remote tissues and organs. Beall and Ulsamer (6) reviewed the literature and concluded that chronic formaldehyde exposure may be associated with hepatotoxicity in many species. Microscopic and biochemical manifestations include centrilobular vacuolization, focal cellular necrosis and increased alkaline phosphatase concentrations. The data in humans are less conclusive; however, isolated reports of acute and chronic formaldehyde exposure and subsequent toxic hepatitis have been reported (7,8). In a rat model, Strubelt et al. (9) demonstrated that the intravenous infusion of formaldehyde (36 mg/kg) produces arterial hypotension, bradycardia, decreased peripheral resistance, acidosis and subsequent death as a consequence of the cardiovascular depressive activity of formaldehyde and its metabolite formic acid. In this study, we used 3 ml of a 10% formalin solution (300 mg, or ~56 mg/kg) without evidence of systemic or cardiovascular toxicity. However, it is unclear what quantity of formalin escapes into the central circulation and what remains within the ductus arteriosus wall and surrounding tissues. In addition, the amount of formalin leading to systemic or cardiovascular toxicity in humans based on body surface area is unknown.

Study limitations. Accurately determining the magnitude of shunting through the ductus arteriosus in an otherwise structurally normal heart is difficult because a true mixed distal pulmonary artery saturation may not be obtainable as a result of streaming. It is recognized that this method may overestimate the degree of left-to-right shunting. However, we used a method comparable to the standard clinical method of sampling in the distal pulmonary artery and the conventional formula for calculating the pulmonary to systemic flow ratio. In general, a "palliative" shunt associated with minimal elevation of pulmonary wedge pressure and an estimated pulmonary to systemic flow ratio of 2:1 would be considered near optimal for surgical palliation. In future studies, serial quantification of left ventricular volumes would further support the pulmonary to systemic flow ratio data. In clinical use, limitation of this technique could be encountered in infants with a tortuous or vertical ductus, making catheter manipulation and transvascular infiltration difficult. In addition, the endothelial lining of the ductus, which appeared regenerated, may become progressively thicker, limiting the effective duration of the procedure. The power of this study is also limited by the small sample size, and thus, additional experiments are necessary to further validate this technique.

Conclusions. For the first time we successfully reestablished and maintained patency of the ductus arteriosus in a small number of newborn lambs by transvascular infiltration and dilation of the ductus arteriosus wall with formalin. This process inhibited constriction of the ductal smooth muscle as a direct result of medial wall destruction, as demonstrated on postmortem histology. The ductus remained patent in all lambs; however, three animals had a less than

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optimal response to balloon dilation with two developing congestive heart failure acutely and one ductus constricting a few weeks after the procedure. In retrospect, alternative diameter balloon dilation catheters based on the animal's weight (smaller diameter balloons for smaller animals) may have avoided these problems. The remaining four lambs had acceptable and consistent pulmonary to systemic flow ratios, ductal caliber and ductal to descending aorta diameter ratios during follow-up and growth of the animals. This would imply that the diameter of the ductus arteriosus increased over time in contrast to the relatively fixed diameter of the conventional "modified" systemic to pulmonary artery shunt generally employed in neonates. We did not appreciate hypotension, bradycardia, hepatic or renal injury following the limited intravascular exposure to formalin. Although transvascular infiltration of the ductus arteriosus may have applications in neonates with certain forms of congenital heart disease, additional experiments are necessary to 1) further validate the technique, 2) determine the minimal amount of infiltrative substance required to maintain ductal patency, 3) find the appropriate balloon diameter based on body surface area or aortic diameter to achieve the desired pulmonary to systemic flow ratio, and 4) ascertain the degree of systemic toxicity of the infiltrative substance.

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